A CELL-BASED ARRAY PLATFORM

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a U.S. national phase application of International PCT Patent Application No. PCT/EP2017/061385, which was filed on May 11, 2017, which claims priority to European Application No. 16169643.0, filed May 13, 2016, each of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is GLYD_001_01US_ST25.txt The text file is 130 KB, was created on July 11, 2019, and is being submitted electronically via EFS-Web.

FIELD OF THE INVENTION

[0003] The present invention relates to a plurality of mammalian cells with different capacities for posttranslational modifications that are useful for displaying and probing biological interactions involving glycans in an arrayable format. The pluralities of mammalian cells are genetically engineered in a combinatorial approach to express different repertoires of glycosyltransferases and interpretable capacities for glycosylation. The plurality of mammalian cells can comprise one or more exogenously added genes encoding polypeptides of interest, wherein the polypeptide of interest is expressed and display different posttranslational modifications in a combinatorial way and dependent on the engineering of the cells. The plurality of engineered cells display glycans with and without the context of specific proteins exogeneously expressed, and is useful for detection of biological interactions for example binding of lectins, antibodies, viruses and bacteria.

[0004] The present invention also relates to methods for generating mammalian cells displaying different glycans, glycoproteins and compositions comprising the glycoproteins, as well as genome engineering, cell-based assays, and their uses.

BACKGROUND OF THE INVENTION

[0005] The glycome of mammalian cells includes all glycans on glycoproteins, glycolipids, proteoglycans and glycosylphosphatidylinositol (GPI) anchored proteins, and comprise a highly diverse set of different glycan structures (Rillahan 2011). The glycome is generated post-translationally through a non-template driven process directed by over 200 glycosyltransferase genes and an equally large number of accessory genes encoding enzymes, transporters, adapters and other proteins required for sugar nucleotide synthesis and transport as well as organization of the glycosylation process in the ER and Golgi complex (Hansen 2015). Differential expression of enzymes and their distinct specificities dictate the unique spectrum of structures produced by a given cell. The glycome of mammalian cells, tissues and organisms play pivotal biological roles in normal and disease states, and many of these roles are directed by protein-carbohydrate and carbohydrate-carbohydrate interactions (Paulson 2006). Many pathogens have glycan-binding-proteins (GBPs) that recognize host glycan structures as receptors for attachment enabling colonization and toxin entry (Sharon 2004, Ilver 2003). Eukaryotic organisms have developed GBPs that recognize pathogen glycans as part of the innate immune system. A large number of mammalian GBPs have also evolved to recognize endogenous host glycans, and GBP receptor interactions mediate a variety of functions in the organism including cell-cell adhesion, trafficking and cell signaling (Taylor 2014). The functions of many mammalian GBPs have been clarified, but there are still many with unknown roles.

[0006] The binding specificity of mammalian and microbial GBPs towards glycans and glycoconjugates has been extensively studied. Microbial GBPs mediate the attachment of microbes and microbial toxins to host cells via cell-surface glycan ligands. The membrane envelopes of for example influenza viruses (and others viruses such as Sendai, Newcastle disease and measles) are studded with hemagglutinins, and these viral GBPs bind to sialic acid-containing glycan ligands to initiate endocytosis (Stevens 2006). Some non-enveloped viruses in the reovirus (rotavirus) families also bind cell-surface sialic acids on host cells through a shallow pocket on the surface of the capsid (Yu 2014). Many bacteria produce adhesins that use glycans for attachment to host cells.

[0007] Progress has been hampered in part by the diversity and complexity of the glycome and technical difficulties in probing interactions. Identification of the fine structural details of ligands for GBPs is confounded by the fact that glycoproteins and glycoconjugates most often carry many different glycan structures as a result of for example heterogeneity and sites of glycosylation, and further that GBPs may selectively recognize glycans in context of the protein or glycoconjugate. Moreover, GBP receptor interactions with ligands may be controlled by particular presentations of the glycoconjugate in cellular systems, such as for example microdomains in cell membranes. The affinity of GBPs for their glycan ligands is typically low (Kd of micromolar to millimolar), and multivalent interactions are required to achieve a biological effect.

[0008] Studies of the binding specificities of GBPs were greatly advanced by the development of glycan-arrays displaying hundreds of homogeneous oligosaccharides produced chemically or chemoenzymatically or isolated from natural sources (Blixt 2004). Most recent versions of glycan microarrays use array printing technologies developed for printing cDNA microarrays on glass slides (Paulson 2006). The method of attachment of the glycan to the solid support and attachment may either be noncovalent or covalent. Several glycan array formats are based on noncovalent association of glycans or modified glycans with appropriately prepared surfaces. The surface to which the glycans are attached is critical for the subsequent interrogation with labeled GBP, as low background binding is essential for specific binding to be detected. Regardless of format, the utility of glycan arrays depends on the types and diversity of glycan structures it contains and limitations governed by the surface and mechanisms of coupling to this surface. The ideal array would contain the entire glycome of an organism on a single array. However, current arrays are limited to displaying libraries of natural and synthetic glycans that can be practically and technically assembled. While glycan arrays have advanced our understanding of the binding